IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Hashime KANAZAWA et al.
Serial No. 10/533,806 Group Art Unit 1625
Filed May 5, 2005
Examiner Mr./Mrs. Rita J. Desai
For: PYRAZOLONAPHTHYRIDINE DERIVATIVES

DECLARATION UNDER RULE 1.132

Honorable Commissioner of Patent and Trademarks, Washington, D.C.

Sir,

I, Kouki Ishitani, the undersigned, a citizen of Japan, residing at 1-8-16-808, Sakae, Soka, Saitama, Japan, do hereby declare:

1. That I am a co-inventor of the above-identified application.

2. That I graduated from Tohoku Pharmaceutical University on March 20, 1989 with a degree in Ph. D.

3. That after working for Ihara Chemical Industry Co., Ltd. as a researcher for 3 years, I joined Grelan Pharmaceutical Co., Ltd. in 1992 and transferred to ASKA Pharmaceutical Co., Ltd. which was formed by a merger of Grelan and Teikoku Hormone Mfg. Co., Ltd. on October 1, 2005, and I have been in charge of planning and conducting pharmacological studies regarding pharmacological active agents under research and

development stage in Grelan and ASKA.

4. That my Relevant Publications are as follows.

1) Phosphodiesterase 4 inhibitor GPD-1116 markedly attenuates the development of cigarette smoke-induced emphysema in senescence-accelerated mice P1 strain. H. Mori, T. Nose, K. Ishitani, S. Kasai, S, Souma, T. Aiyoshi, Y. Kodama, T. Mori, M. Kondo, S. Sasaki, A, Iwase, K. Takahashi, Y. Fukuchi, K. Seyama, Am. J. Physiol. Lung Cell Mol. Physiol., 294, L196-L204,2008.

2) Auranofin inhibits calcium uptake into opsonized-zymosan-stimulated neutrophils obtained from rats. K. Ishitani, A. Matsuura and H. Honda, Inflamm. Res., 44, 482-485, 1995.

3) Xanthine derivatives inhibit the increase of intracellular Ca^{2+} concentration induced by acetylcholine in nasal gland acinar cells of guinea pig. K. Ishitani, K. Ikeda, H. Sunose, D. Wu, H. Honda and T. Takasaka, Eur. Respir. J., 8, 2114-2119, 1995.

4) Intracellular Ca^{2+} response induced by acetylcholine in the submucosal nasal gland acinar cells in guinea pigs. K. Ikeda, M. Ishigaki, D. Wu, H. Sunose, M. Suzuki, K. Ishitani and T. Takasaka, Am. J. Physiol., 268, L361-368, 1995.

5) Effect of trimebutine malate on the contractile response of isolated ileum from diabetic rats. M. Uchida, T. Iwata, Y. Sugiyama, K. Ishitani, H. Honda and Y. Sakai, General Pharmacol., 25, 505-508, 1994.

6) Induction of platelet activating factor in mice by the intravenous administration of a neutral fraction of Bakers' yeast mannan. T. Mikami, K. Fukushi, K. Ishitani, M. Ishitani, S. Suzuki and M. Suzuki, Lipids, 26, 1404-1407, 1991.

7) Influence of transglutaminase on the function of mouse peritoneal macrophages. K. Ishitani and M. Suzuki, Microbiol. Immunol., 33, 59-68, 1989.

8) Influence of arachidonate metabolism on enhancement of intracellular transglutaminase activity in mouse peritoneal macrophages. K. Ishitani, S. Ogawa and M. Suzuki, J. Biochem., 104, 394-402, 1988.

5. That in order to show that the claimed compound has unexpectedly superior PDE IV inhibitory activity and allergic inflammation inhibitory effects and therefore is not obvious over EP 0 526 840 or Suzuki et al. (US 5,281,610), I have performed under my direction and control the following experiments. The particulars and results of the experiments are set forth below.

EXPERIMENT

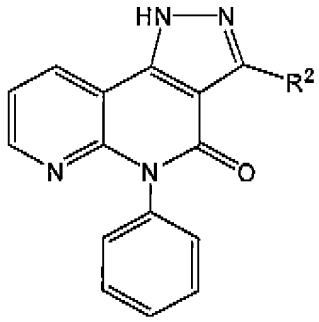
I carried out the following experiments for clearly demonstrating the effects of the present invention.

Assay Example: Inhibitory effects of test compounds in IgE-dependent ear swelling response model mice

In this assay, the test compounds A, B, C, D, and

E shown in Table 1 were used for evaluating allergic inflammation inhibitory effects.

Table 1



Test Compounds	$-R^2$
A	Benzyl
B	Phenyl
C	Methyl
D	Thienyl
E	4-Fluorophenyl

<Protocol>

BALB/cAnNCrlCrlj-series male mice (6 mice per group) were passively sensitized with anti-DNP-IgE antibody (mouse monoclonal anti-dinitrophenyl) in a dose of 0.25 mL/mouse by intravenous administration. On the next day, each test compound (30 mg/kg) was orally administered to the mice of each group in a volume of 10 mL/kg. One hour after the administration, ear swelling was induced by applying 25 μL of 0.15% dinitrofluorobenzene solution (acetone/olive oil = 4/1) to both sides of the right ear of each mouse (challenge). Twenty-four hours after the challenge (late phase), the thickness of the right ear was measured by using a dial thickness gauge.

Incidentally, the thickness of the right ear measured just before the IgE administration was regarded as a pre-value, and the right ear swelling (Δ value) was calculated based on the following equation (1).

$$\Delta T = T - T_0 \qquad (1)$$

wherein T represents the thickness of right ear at measurement time, T_0 represents the pre-value, and ΔT represents the right ear swelling.

Further, the inhibitory rate (%) was calculated based on the following equation (2).

$$\text{Inhibitory rate (\%)} = [(A-B)/A] \times 100 \qquad (2)$$

wherein A represents ΔT in control group, and B represents ΔT in each test group

The results are shown in Tables 2 and 3 and Fig. A.

Table 2 represents the right ear swelling ΔT and the inhibitory rate (%) of each test compound. Table 3 represents a statistical analysis in the right ear swelling ΔT between the test compound A and each of the test compounds B, C, D and E.

Fig. A represents the right ear swelling ΔT of the test compounds A-E in IgE-dependent ear swelling response model mice. The numerical values in the figure represent the inhibitory rate (%) calculated based on the equation (2) in each of the test compounds. The symbol "*" represents that there is a significant difference ($P<0.05$) in the right ear swelling ΔT

between the each test compound and the control; the symbol "**" represents that there is a significant difference ($P<0.01$) in the right ear swelling ΔT between each test compound and the control.

The symbol "*" represents that there is a significant difference ($P<0.05$) in the right ear swelling ΔT between the test compound A and the test compound B; and the symbol "**" represents that there is a significant difference ($P<0.01$) in the right ear swelling ΔT between the test compound A and each of the test compounds C,D and E.

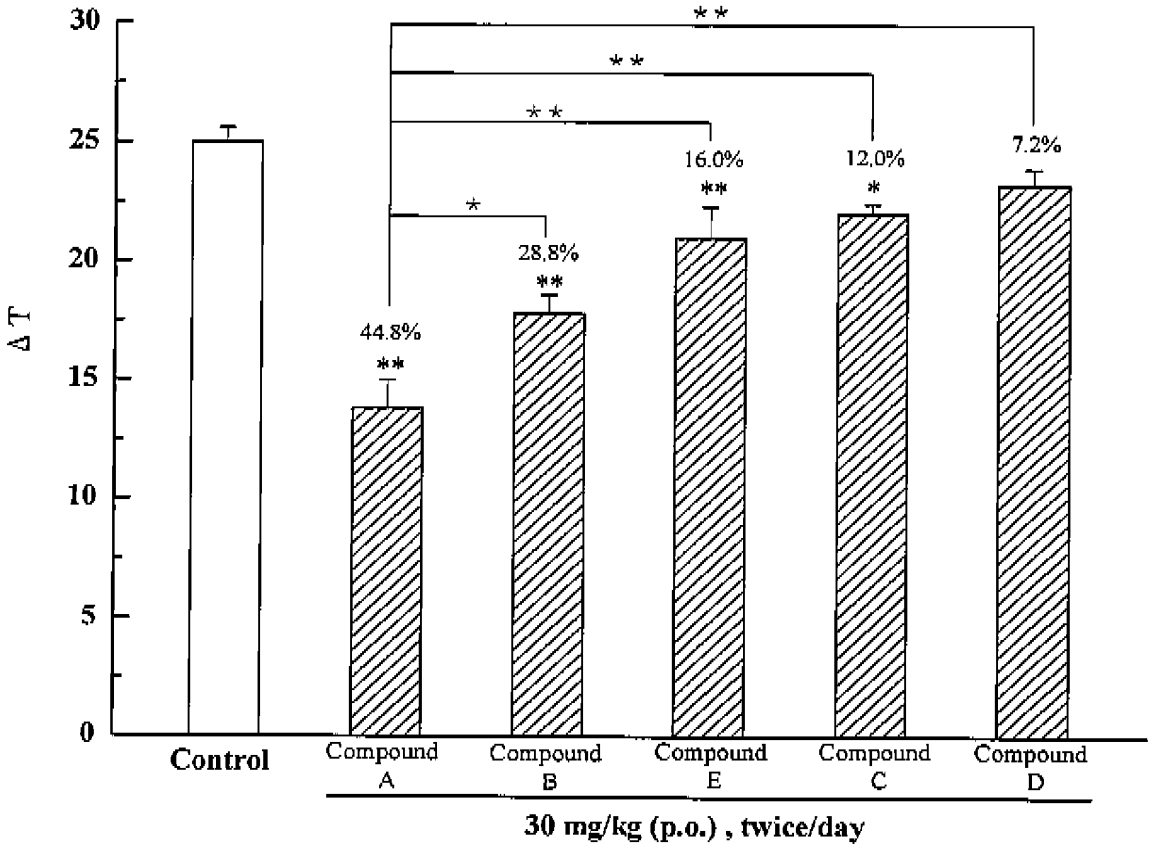
Table 2 (ΔT)

	ΔT		Inhibitory rate (%)
	Mean	SE	Mean
Control	25.0	0.6	
Compound A	13.8	1.2	44.8
Compound B	17.8	0.8	28.8
Compound E	21.0	1.3	16.0
Compound C	22.0	0.4	12.0
Compound D	23.2	0.7	7.2

Table 3

Method	vs	P value	
Dunnett	Compound A vs Compound B	0.0112	* (P<0.05)
Dunnett	Compound A vs Compound E	3.30307×10^{-5}	** (P<0.01)
Dunnett	Compound A vs Compound C	6.22227×10^{-6}	** (P<0.01)
Dunnett	Compound A vs Compound D	1.95488×10^{-6}	** (P<0.01)

Fig. A



As apparent from the results, the test compound A (that is, the compound of the present invention) revealed stronger inhibitory effects on IgE-dependent late-phase inflammation, compared with other test compounds B, C, D, and E (that is, compounds described in US Patent No. 5,281,610). This assay is one of tests for confirming allergic inflammation inhibitory effects, and the results proved that the compound of the present invention effectively inhibits allergic inflammation.

Preparation of Test Compounds

Test Compound A: 3-Benzyl-5-phenyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one)

Test Compound A corresponds to the compound of Example 9 of this description. The test compound A was prepared according to the procedure described in Example 9 of this description.

(A-1) Synthesis of 4-hydroxy-3-(1-oxo-2-phenylethyl)-1-phenyl-1,8-naphthyridin-2(1H)-one

(i) A mixture of 4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one (1.19 g, 5.0 mmol; synthesized according to JP, A, 61-246183 (1986)) and sodium hydride (about 60%, 200 mg, 5.0 mmol) was added DMF (10 ml), and the resultant mixture was stirred to form a solution until the production of hydrogen was completed. Next, after phenylacetyl chloride (0.8 ml, 6 mmol) was added, the mixture was stirred for 1 hour at 50°C, treated with saturated aqueous sodium hydrogen carbonate, and filtered off to give precipitates which were washed with water and dried to afford 1-phenyl-4-phenylacetoxy-1,8-naphthyridin-2(1H)-one.

(ii) To a mixture of 1-phenyl-4-phenylacetoxy-1,8-naphthyridin-2(1H)-one (1.34 g, 3.7 mmol), triethylamine (379 mg, 3.7 mmol), potassium cyanide (491 mg, 7.5 mmol), and 18-crown-6 (197 mg) was added dry toluene (35 ml), and the mixture was stirred at room temperature overnight, admixed with saturated aqueous sodium hydrogen carbonate and dichloromethane. The organic layer was washed with saturated aqueous

sodium hydrogen carbonate, and successively with saturated aqueous sodium chloride. The organic layer was collected, dried over anhydrous magnesium sulfate, and evaporated. The resulting residue was purified by flash column chromatography to afford 4-hydroxy-3-(1-oxo-2-phenylethyl)-1-phenyl-1,8-naphthyridin-2(1H)-one as crystals.

(A-2) Synthesis of 3-benzyl-5-phenyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one)

To a suspension of 4-hydroxy-3-(1-oxo-2-phenylethyl)-1-phenyl-1,8-naphthyridin-2(1H)-one (2.33 g, 6.54 mmol, prepared in the above step (A-1)) in DMF (50 ml) was added hydrazine monohydrate (80%, 970 µl, 24.21 mmol, 3.7 eq.) and the mixture was then stirred at 100 to 110°C for 4 hours, admixed with water to precipitate crystals, and allowed to stand until it was cooled. Next, the cooled mixture was filtered, washed with water, and dried to give 3-benzyl-5-phenyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one.

Test Compound B: 3,5-Diphenyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one

Test Compound B corresponds to Compound 1 of US Patent No. 5,281,610. The test compound B was prepared according to US Patent No. 5,281,610.

Benzoyl chloride, 1.75 ml (15.1 millimoles), was added to nitrobenzene (30 ml) containing 5.0 g (37.8 millimoles) of aluminum chloride under an argon atmosphere, and the mixture was stirred for 1 hour at

room temperature to form a uniform solution. To the solution was added 3.0 g (12.6 millimoles) of 4-hydroxy-1-phenyl[1,8]naphthyridin-2(1H)-one, and the mixture was stirred at 160° to 170°C for 6 hours. Dilute hydrochloric acid was added to the reaction mixture, and the mixture was extracted with chloroform. The extract was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the crude product was recrystallized from dimethylformamide (DMF)-water to obtain 3-benzoyl-4-hydroxy-1-phenyl[1,8]naphthyridin-2-(1H)-one.

850 mg (2.5 millimoles) of 3-benzoyl-4-hydroxy-1-phenyl[1,8]naphthyridin-2-(1H)-one was suspended in 5 ml of glacial acetic acid, and 0.26 ml (5.5 millimoles) of hydrazine monohydrate was added to the suspension. The mixture was heated under reflux for 5 hours and the reaction mixture was cooled to room temperature. The precipitate was collected by filtration, and recrystallized from DMF-water to obtain Test Compound B.

Test Compound C: 3-Methyl-5-phenyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one

Test Compound C corresponds to Compound 2 of US Patent No. 5,281,610. The test compound C was prepared according to US Patent No. 5,281,610.

4-Hydroxy-1-phenyl[1,8]naphthyridin-2(1H)-one, 20.0 g (84.0 millimoles), was added to 170 ml of glacial acetic acid and 340 ml of polyphosphoric acid. The mixture was stirred at 100° to 110°C for 5 hours.

The reaction solution was poured into water and the mixture was stirred for 1 hour. The precipitate was collected by filtration, and recrystallized from DMF-water to give 3-acetyl-4-hydroxy-1phenyl[1,8]naphthyridin-2(1H)-one.

3-Acetyl-4-hydroxy-1phenyl[1,8]naphthyridin-2(1H)-one in an amount of 1.2 g (4.3 millimoles) was suspended in 10 ml of glacial acetic acid, and 0.46 ml (9.4 millimoles) of hydrazine monohydrate was added to the suspension. Thereafter, the mixture was heated under reflux for 3 hours. The reaction solution was cooled to room temperature, and the precipitate was collected by filtration and recrystallized from DMF-water to give Test Compound C.

Test Compound D: 5-Phenyl-3-thienyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one

Test Compound D corresponds to Compound 10 of US Patent No. 5,281,610. The test compound D was prepared according to US Patent No. 5,281,610.

(D-1) Synthesis of 1-phenyl-2H-pyrido[2,3-d][1,3]oxazine-2,4m(1H)-dione

7.0 g (0.031 mole) of methyl 2-anilinonicotinate (J. Org. Chem., Vol. 39, page 1803, 1974) was dissolved in a mixture of 70 ml of 1,2-dichloroethane and 7 ml of dioxane. With stirring at 60°C, 11 ml (0.092 mole) of trichloromethyl chloroformate was added to the solution. The mixture was refluxed for 3 hours. After slight cooling, 0.25 g of activated charcoal was added to the

reaction mixture, and the reaction mixture was further refluxed for 30 minutes in a stream of nitrogen. The mixture was cooled to room temperature, filtered and concentrated. The precipitated crystals were recrystallized from dichloromethanediisopropyl ether to give 1-phenyl-2H-pyrido[2,3-d][1,3]oxazine-2,4m(1H)-dione as a colorless crystals.

(D-2) Synthesis of 3-cyano-4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one

1.6 ml (0.020 mole) of ethyl cyanoacetate was dissolved in 25 ml of N, N-dimethylacetamide, and 0.80 g (0.020 mole) of 60% sodium hydride was added with ice cooling. After hydrogen ceased to be evolved, 4.0 g (0.017 mole) of 1-phenyl-2H-pyrido[2,3-d][1,3]oxazine-2,4m(1H)-dione obtained in the above step (D-1) was added. The mixture was gradually warmed, and stirred at 100°C for 30 minutes. The solution was cooled to room temperature, the solvent was evaporated under reduced pressure and water was added. The mixture was washed with ethyl acetate, the aqueous layer was acidified with concentrated hydrochloric acid, and the resulting crystals were collected by filtration. The crystals were recrystallized with ethanol to give 3-cyano-4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one.

(D-3) Synthesis of 5-phenyl-3-thienyl-1H-pyrazolo[4,3-c][1,8]naphthyridin-4(5H)-one

In an argon atmosphere, 5.0 g (19.0 millimoles) of 3-cyano-4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one obtained in the above step (D-2) was suspended in

100 ml of tetrahydrofuran. 2-Thienyl magnesium bromide (60.8 millimoles) in tetrahydrofuran was added to the suspension under ice cooling. The mixture was stirred at 60°C for one hour, and dilute hydrochloric acid was added to the reaction mixture. The mixture was heated under reflux for 5 hours. The reaction solution was cooled to room temperature. Then, the precipitate was collected by filtration, and recrystallized from DMF-water to give 4-hydroxy-1-phenyl-3-thenoyl[1,8]naphthyridin-2-(1H)-one.

4-Hydroxy-1-phenyl-3-thenoyl[1,8]naphthyridin-2-(1H)-one, 2.0 g (5.7 millimoles), was suspended in 60 ml of glacial acetic acid, and 0.42 ml (8.6 millimoles) of hydrazine monohydrate was added to the suspension. The mixture was heated under reflux for 6 hours. The reaction mixture was cooled to room temperature and the precipitate was collected by filtration. Recrystallization from DMF-water gave Test Compound D.

Test Compound E: 5-Phenyl-3-(4-fluorophenyl)-1H-pyrazolo[4,3-c][1, 8]naphthyridin-4(5H)-one

Test Compound E corresponds to Compound 13 of US Patent No. 5,281,610. The test compound E was prepared according to US Patent No. 5,281,610.

In an argon atmosphere, 3.5 g (13.3 millimoles) of 3-cyano-4-hydroxy-1-phenyl-1,8-naphthyridin-2(1H)-one obtained in the above step (D-2) was suspended in 70 ml of tetrahydrofuran. 4-Fluorophenylmagnesium bromide (29.3 millimoles) in tetrahydrofuran was added

to the suspension under ice cooling. The mixture was stirred at room temperature for 2.5 hours, dilute hydrochloric acid was added to the mixture, and the solution was heated under reflux for 8 hours. The reaction mixture was cooled to room temperature, and the precipitate was collected by filtration, and recrystallized from DMF-water to obtain 4-hydroxy-1-phenyl-3-(4fluorophenyl)[1,8]naphthyridin-2(1H)-one.

4-Hydroxy-1-phenyl-3-(4fluorophenyl)[1,8]naphthyridin-2(1H)-one, 2.5 g (6.9 millimoles), was suspended in 50 ml of glacialacetic acid. After adding 0.40 ml (8.3 millimoles) of hydrazine monohydrate to the suspension, the mixture was heated under reflux for 2 hours. The reaction mixture was cooled to room temperature, and the precipitate was collected by filtration and recrystallized from DMF-water to give Test Compound E.

EVALUATION

As apparent from the above results, the compound of the present invention (Test Compound A) has superior allergic inflammation inhibitory effects, compared with the compounds described in US Patent No. 5,281,610.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 9th day of April, 2009

Kouki ISHITANI